



Western Regional Research Center
Albany, CA



“Salmonella Summit”, June 28, 2006
Beltsville, MD

“Enumeration of *Salmonella*”
and
“Potential new methods to genotype *Salmonella*”

Robert Mandrell
Research Leader

Produce Safety and Microbiology Research Unit
USDA, ARS, Western Regional Research Center
Albany, CA

Salmonella and issues relevant to:

- Enumeration (M. Brandl, ARS-PSMRU)
- Affordable rapid real-time PCR for food producers (J. Barak (ARS-PSMRU) and M. Delwiche (UCD))
- Genotyping and strain relatedness
 - Microarrays (C. Parker, ARS-PSMRU)
 - High-resolution SNP arrays (G. Andersen, LBL)

“Enumeration”

Our goal is to quantify accurately the number of viable *Salmonella* cells on a food sample?

Bacteria in complex samples often form aggregates

A “Colony Forming Unit” isolated by plating a complex sample (e.g. food, leaf, root tissue, soil, water) seldom represents a single viable bacterial cell

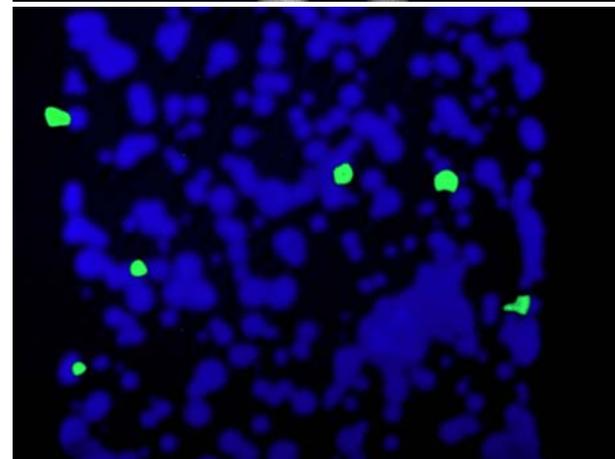
C. jejuni cells aggregated
on chicken skin after short
incubation



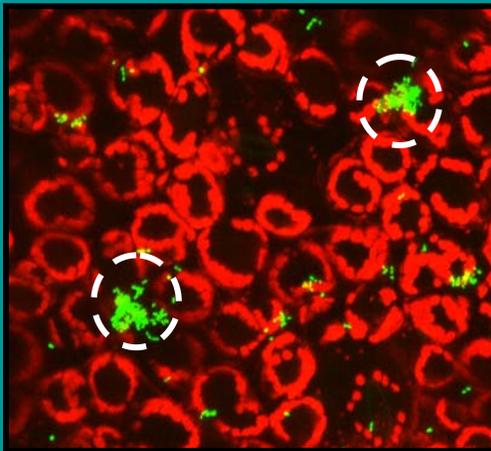
QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.



C. jejuni “CFU” resulting
from chicken tissue
samples plated on agar



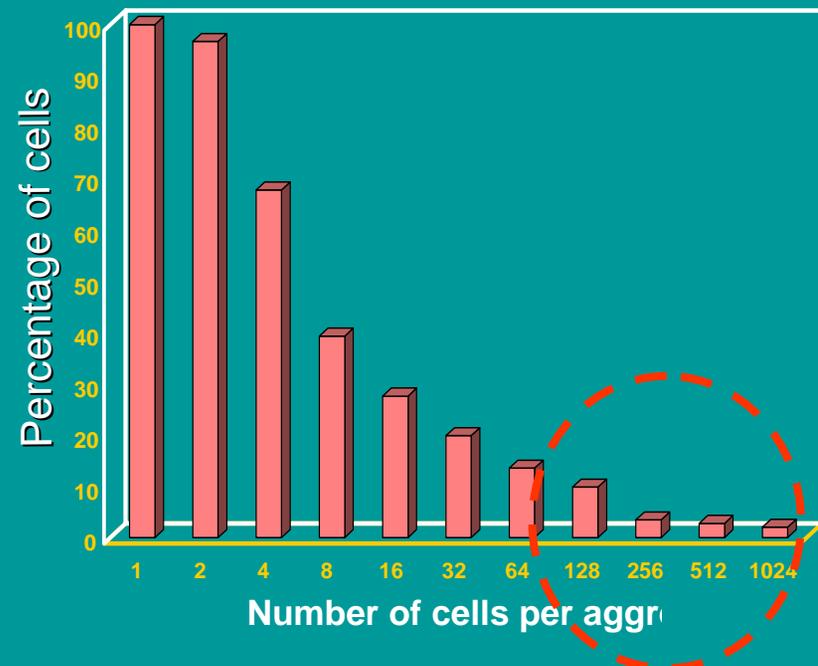
Salmonella aggregates washed from healthy leaves



Aggregates remaining on leaf after sonication and vortexing



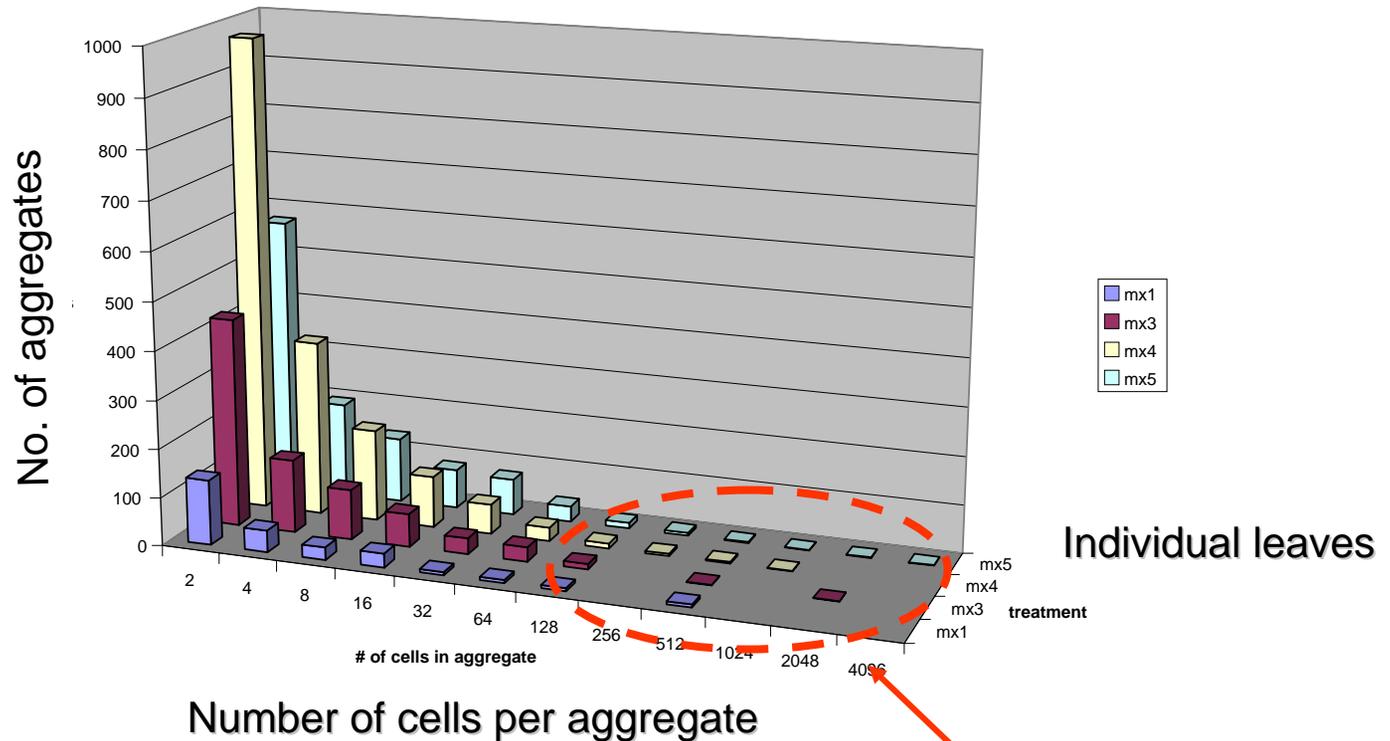
Aggregates in leaf washings after sonication and vortexing



10% of cells in wash solution located in aggregates of >128 cells:

Aggregates = a single cfu

Frequency distribution of *Salmonella* aggregate size on cilantro leaves



54% of total *Salmonella* cell population on leaves

Summary

- Bacterial cells grown on plant tissue are different physiologically than cells cultured in laboratory media.
- Sanitizers should be tested on cells in a state similar physiologically to cells in the environment in which the sanitizers will be used.

Inexpensive PCR system for food processors

(J. Barak and M. Delwiche)

PCR based biosensor for *Salmonella*

collaboration J. Barak (ARS-PSMRU) - M. Delwiche (UCD)

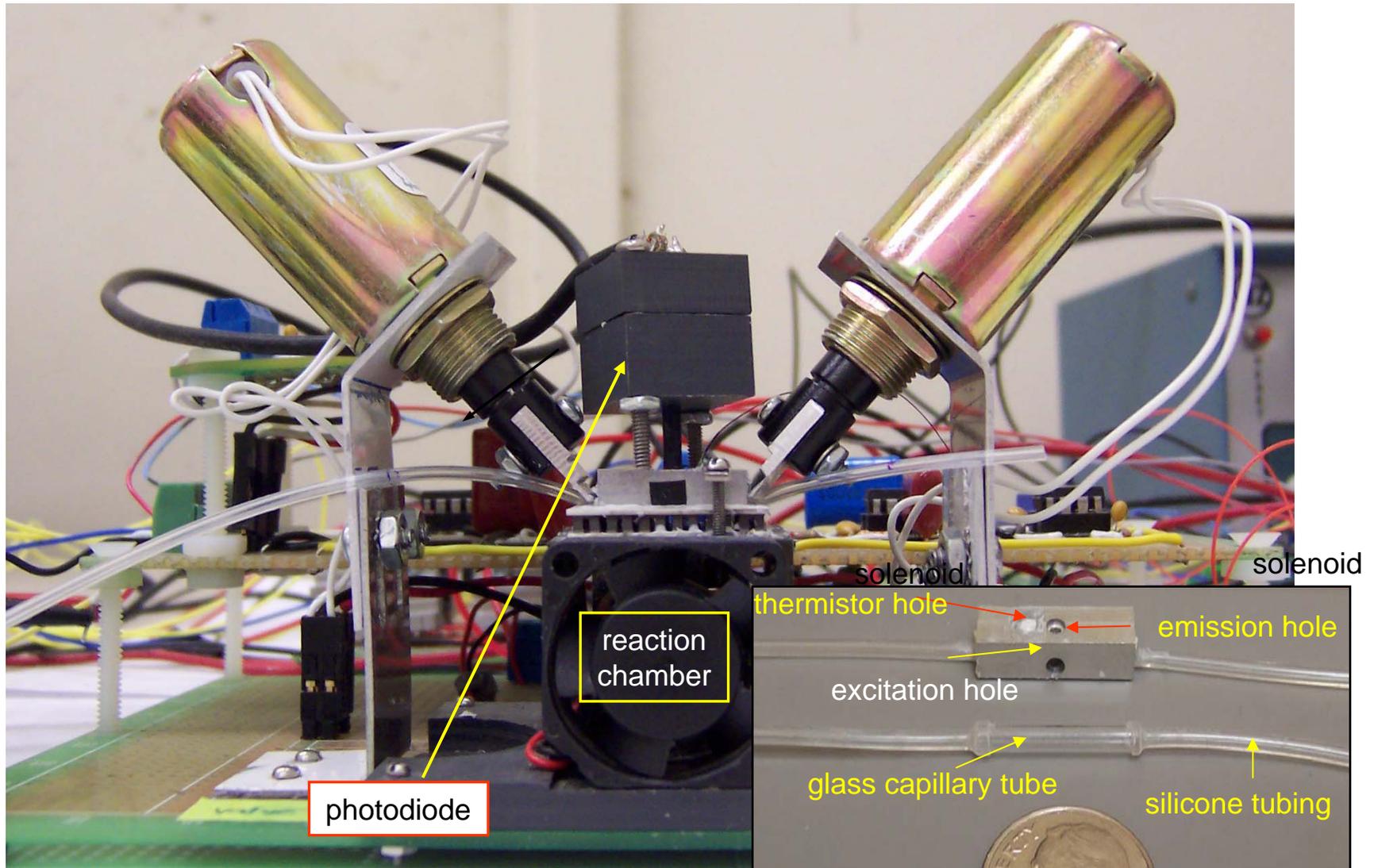
Objective

- Try to build a biosensor to be used in produce packing house or sprout facility
 - Inexpensive
 - Sensitive
 - Specific
 - Rapid: no enrichment, real-time results

Objectives for a 2nd generation biosensor

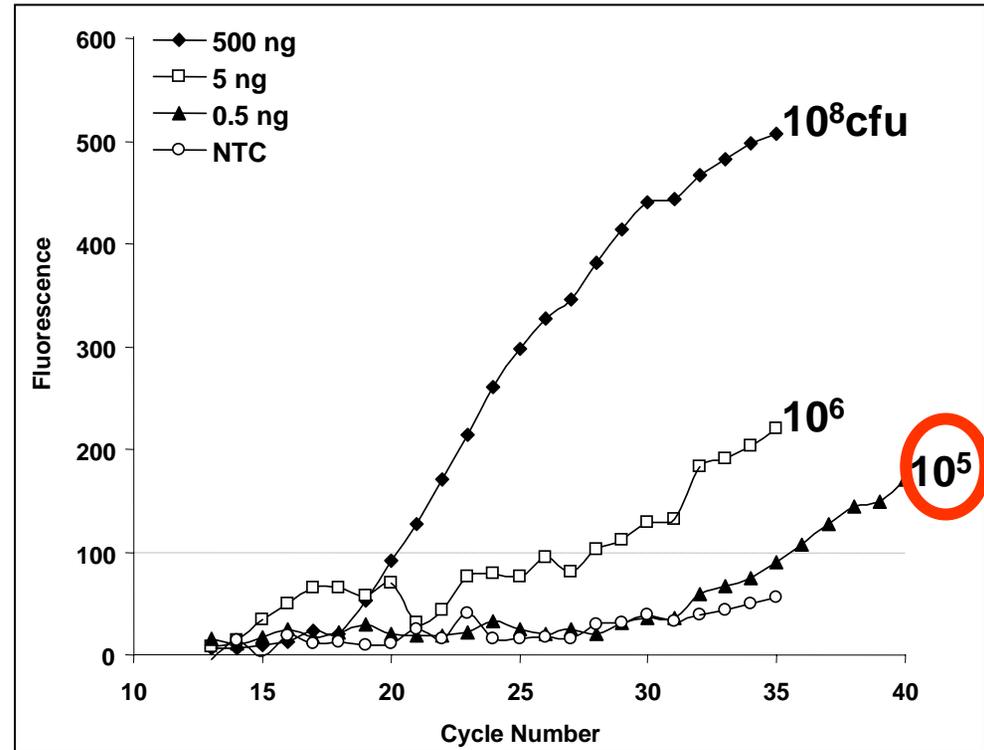
- More sensitive fluorophore and optics
 - Increase sensitivity to 10-100 cfu
- Completely integrated system
 - Automated
 - Can be operated by non-technical staff

Second generation prototype



Completed

- Fully automated system
- Small footprint
- Rapid
- No enrichment
- 2.5 hours run time
- Inexpensive, ~\$1000



Improvements in sensitivity ongoing

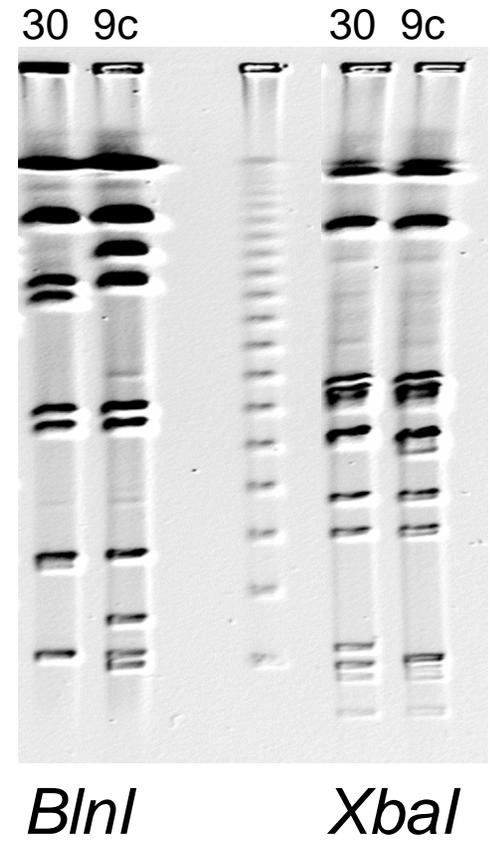
Genotyping

Methods

- Current methods
 - Serotyping and phage typing: require special reagents and reference labs
 - PFGE: useful for identical strains, i.e. outbreaks; not as good for determining strain relatedness (phylogeny)
- Genotyping; sequence-based methods conducive to development of databases and determining relatedness (phylogeny)
 - MLST
 - MLVA
 - Microarrays
 - SNP arrays

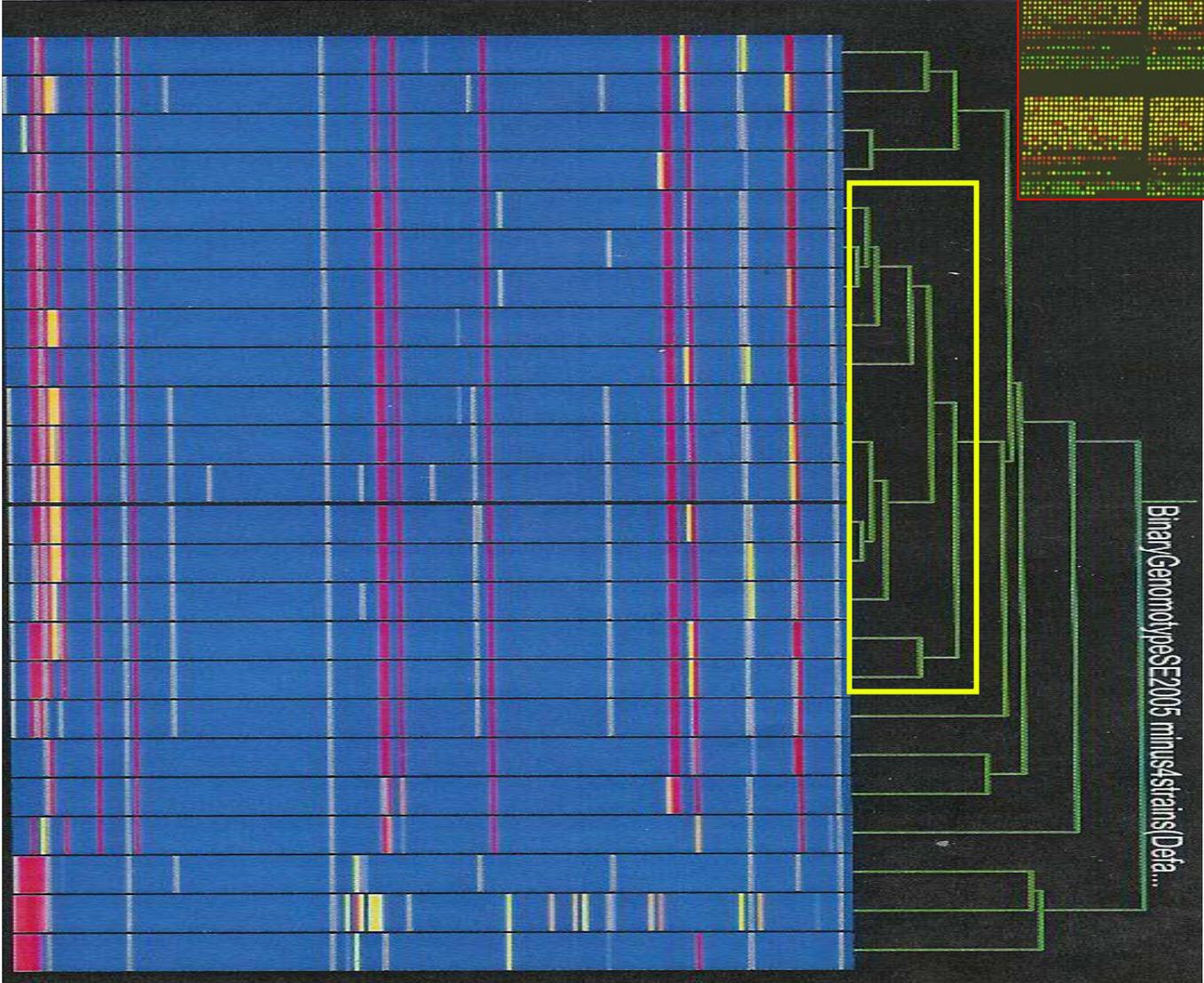
S. Enteritidis: Example of importance of genotyping

- Two Outbreaks of SE associated with raw almonds
 - Outbreak 1: 2000-01
 - Identified by Canada
 - Multi-state and Canada
 - >50 cases
 - SE PT30
 - Outbreak 2: 2004
 - Identified by Oregon DPH
 - Multi-state and Canada
 - >40 cases, 1 death
 - SE PT9c
 - PT30 and PT9c are very rare PTs
- **Different PFGE profiles and PTs:**
Are strains of the two PTs related?



Salmonella microarray: gene-indexing (>3000 gene features)

SE PT33
SE PT33
SE PT8
SE PT13a
SE PT30-Env
SE PT30-Env
SE PT30-Env
SE PT30-Env
SE PT30-Env
SE PT30-Env
SE PT30-Hu-Out1
SE PT30-Hu-Out1
SE PT30-Alm
SE PT30-Hu-Out1
SE PT30-Hu-Out1
SE PT9c-Hu-Out2
SE PT9c-Hu-Out2
SE PT6a
SE PT4
SE PT4
SE PT?
STyph
STyph
STyph



Microarray gene-indexing of SE PT30 vs. SE PT9c

- >3000 genes on *Salmonella* microarray
- SE PT30 and SE PT9c are highly related to one another compared to other SE and much less related to *S. Typhimurium*
- The difference in 9c and 30 may be simply a “wild” phage insertion into 9c
 - This could result in a modified PFGE pattern

Genotyping *Salmonella* by Single
Nucleotide Polymorphisms
("SNP Arrays")

SNP Array

- SNPs should be distributed throughout genome.
- SNPs can differentiate strains of *Salmonella*.
- Provides strain-relatedness data for:
 - Source-tracking.
 - Study evolution of the pathogen.



Salmonella SNP array



- The SNP discovery and genotyping were accomplished using oligo microarrays prepared by Perlegen Sciences, Inc. (<http://www.perlegen.com>) in collaboration with G. Andersen, LBL.
- **SNP discovery:** Re-sequence every base in both forward and reverse strand
 - **60 million probe array** produced from analysis of 4 genomes:
 - S. Typhi CT18, S. Typhi Ty2, S. Enteritidis PT4 and S. Typhimurium LT2
 - Genomic DNA purified, end-labeled with biotin and hybridized to a array
- **Genotyping with SNPs:** SNP discovery facilitated design of a **500,000-probe** genotyping array and testing with 217 strains of *S. Enteritidis*, Typhi, Dublin, Typhimurium, others.
- **Data analysis:** **33,286 SNPs** total analyzed and mapped to at least one of the four reference genomes.
- **Genomic deletions revealed:** (oligoNT hybridization at 10 adjacent SNP loci)

Conclusions

- SNPs identified are evenly distributed throughout genomes
- SNPs are powerful for grouping *Salmonella*
- Deletions detected
- Non-synonymous SNPs are more variable in distribution
- Although massive SNP arrays are not accessible easily (\$\$), the data can be used to develop simpler methods